Module 1: Space Biotechnology
Cellular Systems

Neal R. Pellis, Ph.D.
Biological Systems Office
NASA Johnson Space Center
Houston, TX 77058
281-483-2357
Npellis@ems.jsc.nasa.gov

The Human Experience in Microgravity

- Cardiovascular Deconditioning
- Gastrointestinal Distress
- Bone resorbtion
- Muscle Atrophy
- Orthostatic Intolerance
- Fluid Shifting
- Vestibular Disturbances
- Immune Dysfunction
- Renal stones
- Delayed wound healing
- Exposure to ionizing radiation
- Psychosocial impacts

Biotechnology

- Use of living systems or derivatives of living systems to develop or operate a product or service

Space Biotechnology

- Use of microgravity and space technologies to advance biotechnological strategies

Space Biotechnological Strategies

- Diffusion limited crystallization
  - Materials Science
  - Protein and macromolecular structure
- Electrophoresis
  - Minimizes convective interference with separation
  - May afford novel approaches to cell separation and purification
- Cell culture
  - Minimizes cell interaction with inert surfaces
  - Three dimensional freedom
  - Potential novel response suite

Paradigms Lost

- The Earth is the center of the universe
- Blood letting ameliorates most disease
- Accumulations of old rags in the attic spontaneously generate rats
- Read my lips, no new taxes
- Cellular and intracellular components are too insignificant in mass to be affected by the loss of gravity

Interactions in Nature

- Gravitational
- Electromagnetic
- Strong submolecular forces
- Weak submolecular forces

Interactions in Nature

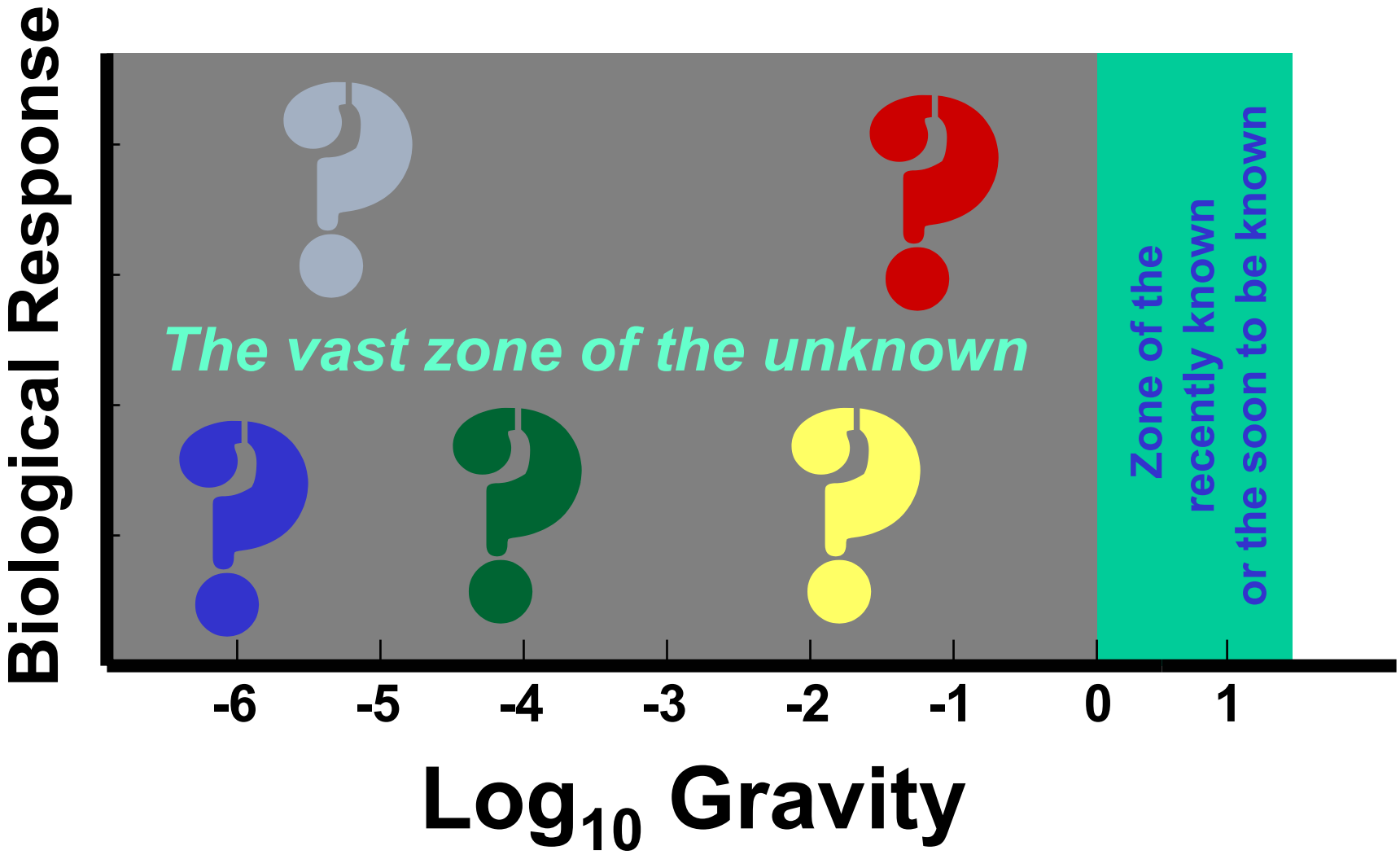
- Gravity is the weakest of the four but has a huge radius of operation
- Among the four it is considered the sculptor of the universe
- Methods for studying gravitational influences on biological processes (Microgravity Analogs)
  - Theoretical analysis and computer modeling
  - Changing the weight loading
  - Hypergravity
  - Free fall strategies

  - *Space experiments*

Definitions

- **Gravity**- force which attracts all objects near the earth towards its center (9.8m/sec^2) or 1*g*
- **Hypogravity or Fractional Gravity**- Less than 9.8m/sec^2
- **Microgravity**- ~10^{-6}*g*
- **"0" gravity**- erroneous term used instead of 'microgravity', frequently used in the media
- **Free Fall**- allowing objects to achieve terminal velocity
- **Drop tower**- 2-5 sec free fall model
- **Parabolic Flight**- 20-25 sec free fall model
- **Sounding Rocket**- free fall for 3-11 min
- **Antiorthostatic suspension**- animal model for cephalad fluid shift experienced in microgravity
- **Bedrest**- human model for microgravity
- **Isolation**- behavioral model for space travel
- **Hardware**- NASAese for equipment
- **Cell Bioreactor**- free fall and randomized *G* model for cells and cell culture
- **Countermeasures**- actions taken to ameliorate effects of space flight

The Basic Problem in Space Cell Biology



Scientific Questions to Address

- Adaptive responses of cells to microgravity and to the space environment?
- Phenotypic and genotypic changes induced by microgravity, space, and planetary environments?
- Does the space environment invoke a selective pressure on replicating cells?
- What applications are unique to low gravity environments?

Implementation Questions to Address

- How to conduct focused programs of investigation in space?
- What are the appropriate analogs for μG to use in cell biology experiments?
- What are the controls that maximize the opportunity to ascribe a biological phenomenon to the influence of μG?

Areas of Impact for Space Exploration

- Basic human physiology
- Plant life used for O_2 for food
- Bioregenerative microbes
- Normal flora
- Environmental monitoring

Areas of Impact for Applied Science

- Tissue engineering
- Vaccine and drug development
- Models of human disease
- Biosensors

Potential Impact on Cells

- Shape
- Signal transduction
- Cell division
- Gene expression
- DNA damage
- Orientation of subcellular components
- Programmed cell death
- Cellular movement
- Synthesis and orientation of macromolecules
- Cellular repair
- Cytokine synthesis and secretion
- Glycosylation
- Biofilm formation

History of Space Cell Biology

- Hyper G experiments on plants and oocytes in 1806 and 1883
- Early satellites of the 1960's had bacterial plant and animal cells- confounded by poor controls
- Skylab- early studies on the human red blood cell shape and metabolic changes; immune cell performance in vitro
- From Skylab to 1995 the cell based research in μG was a diverse collection of experiments that set the format for modern Program oriented approach, i.e. gene expression

Changes in Cell Function under Microgravity Conditions

Cell Type	*Altered Cell Function*
	Energy Source Consumption
WI 38 (human)	20% reduction of glucose uptake
	Biosynthesis*
Human Lymphocytes	Five-fold increase of α-interferon production
	Differentiation**, †
Human Lymphocytes	90% reduction in activation by con A
	Conjugation†
Escherichia-coli	Up to 40% increase in DNA transfer
	Resistance to Antibiotics**,†
Escherichia-coli	Increased resistance
	Growth Rate and Yield†
Paramecium tetraurelia *Chlamydomonas sp.,* *Bacillus subtilis,* *Anise cells*	Increased growth rate and higher yield
	Intracellular Transport
Physarum *polycephalum*	Increased frequency of oscillations and Velocity of cytoplasmic streaming velocity

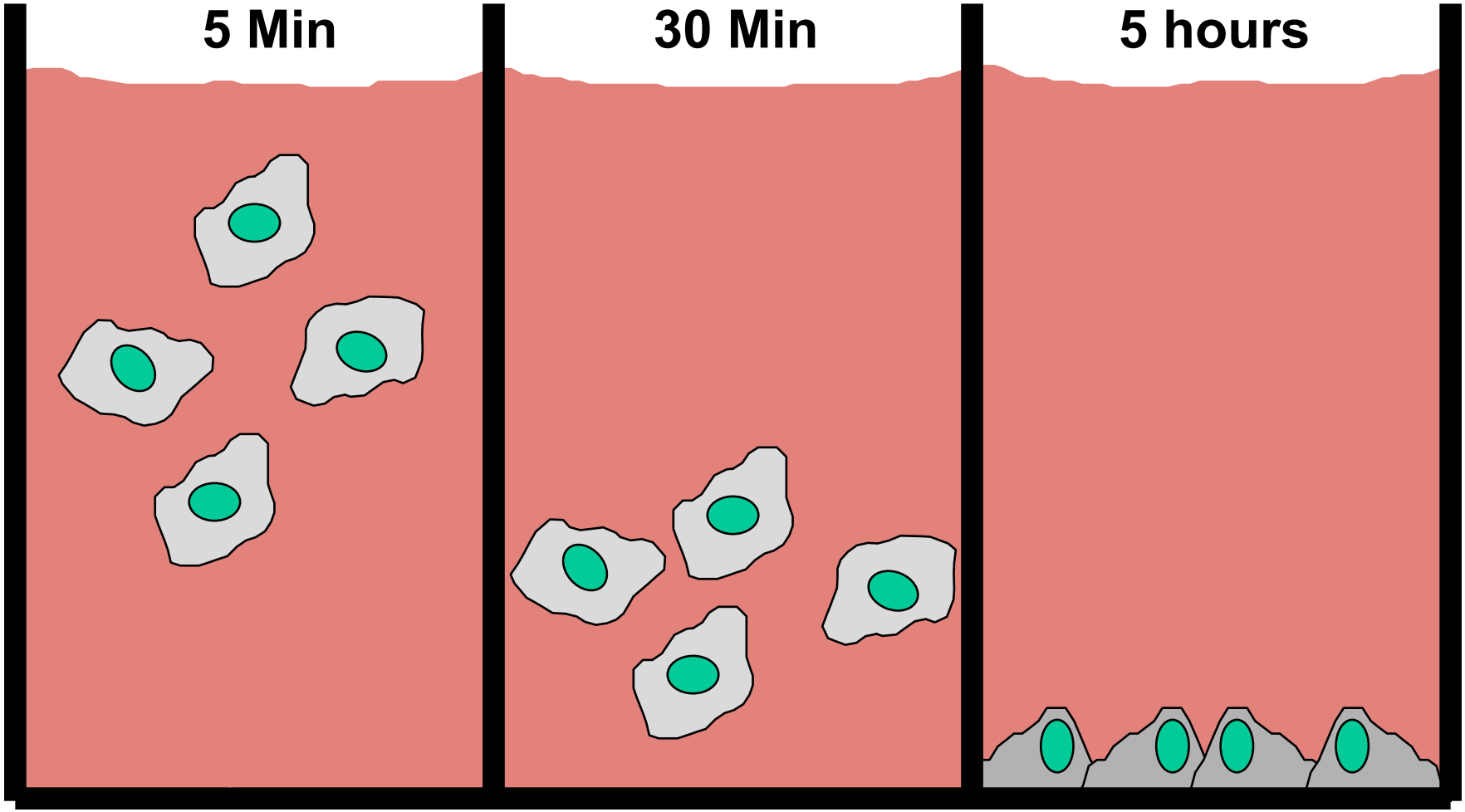
* Results obtained from independent experiments on at least two different space missions.

† Experiments with an in-flight 1-G control.

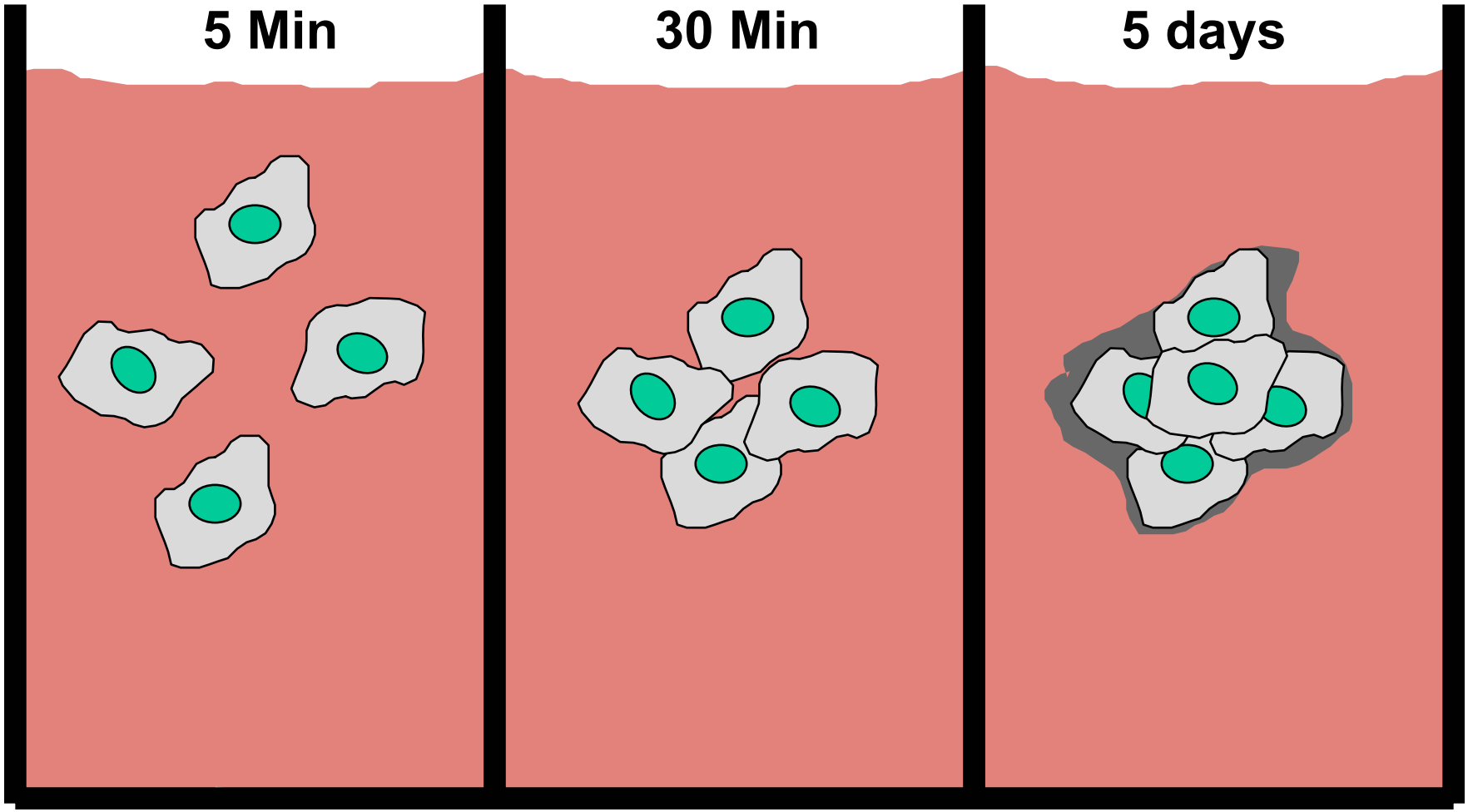
Mechanism of gravity induced cellular changes

- Early theories
  - Molecules or organelles serve as gravity sensors
  - Physicochemical effect leading to adaptation by the cell
  - Holistic bifurcation theory- binary on/off response by the cell
- Emerging theories
  - Loss of gravity results in a reordering of the priority of other forces (intermolecular)
  - Physical effect is singular initiator of a cascade changes in cell shape, organelle orientation, and membrane architecture
  - Physical effects change culture conditions- convection, mass transfer, boundary conditions

1G Cell Culture



Cell Culture in μG



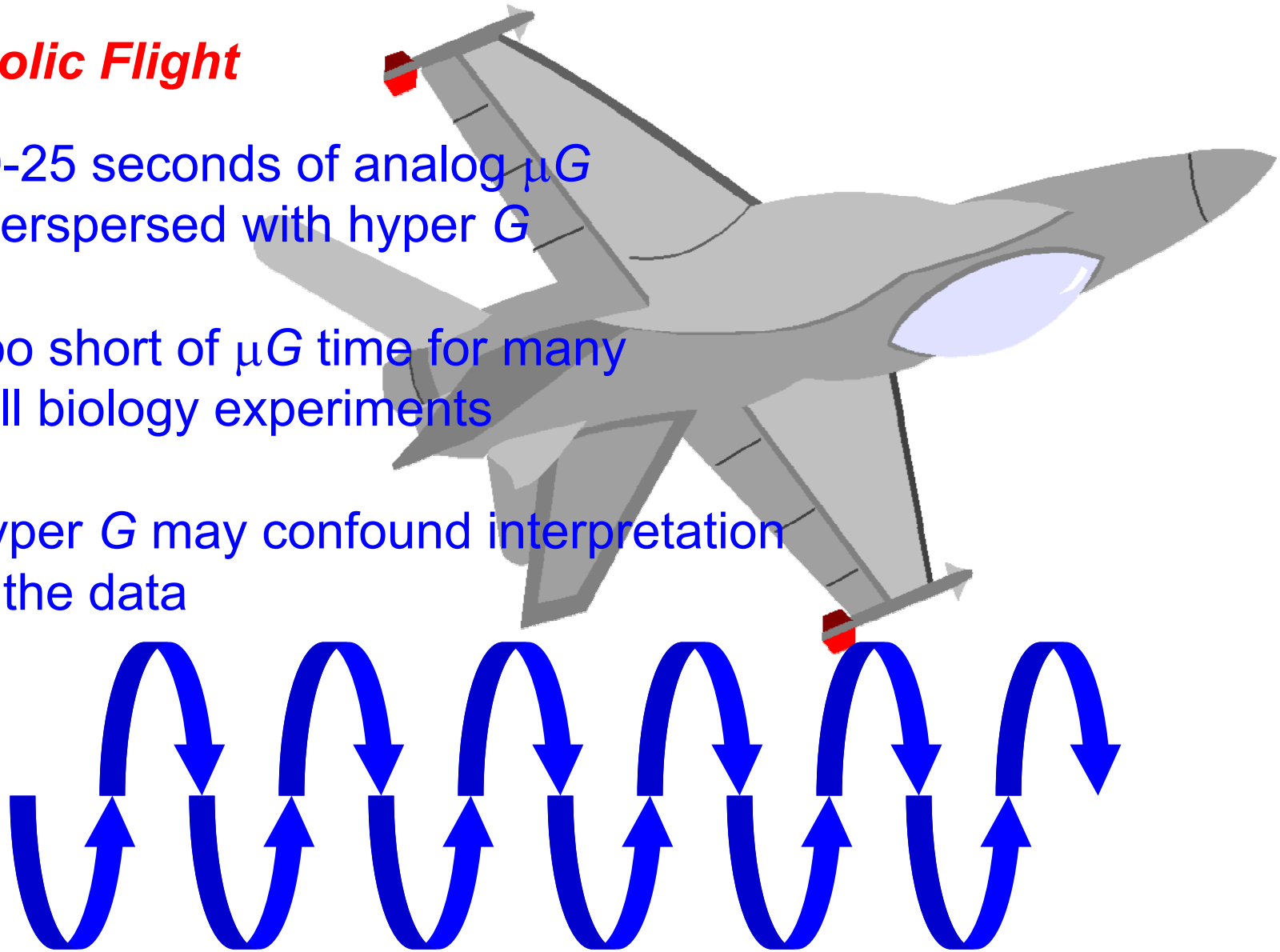
Microgravity Analogs

Parabolic Flight

20-25 seconds of analog μG interspersed with hyper *G*

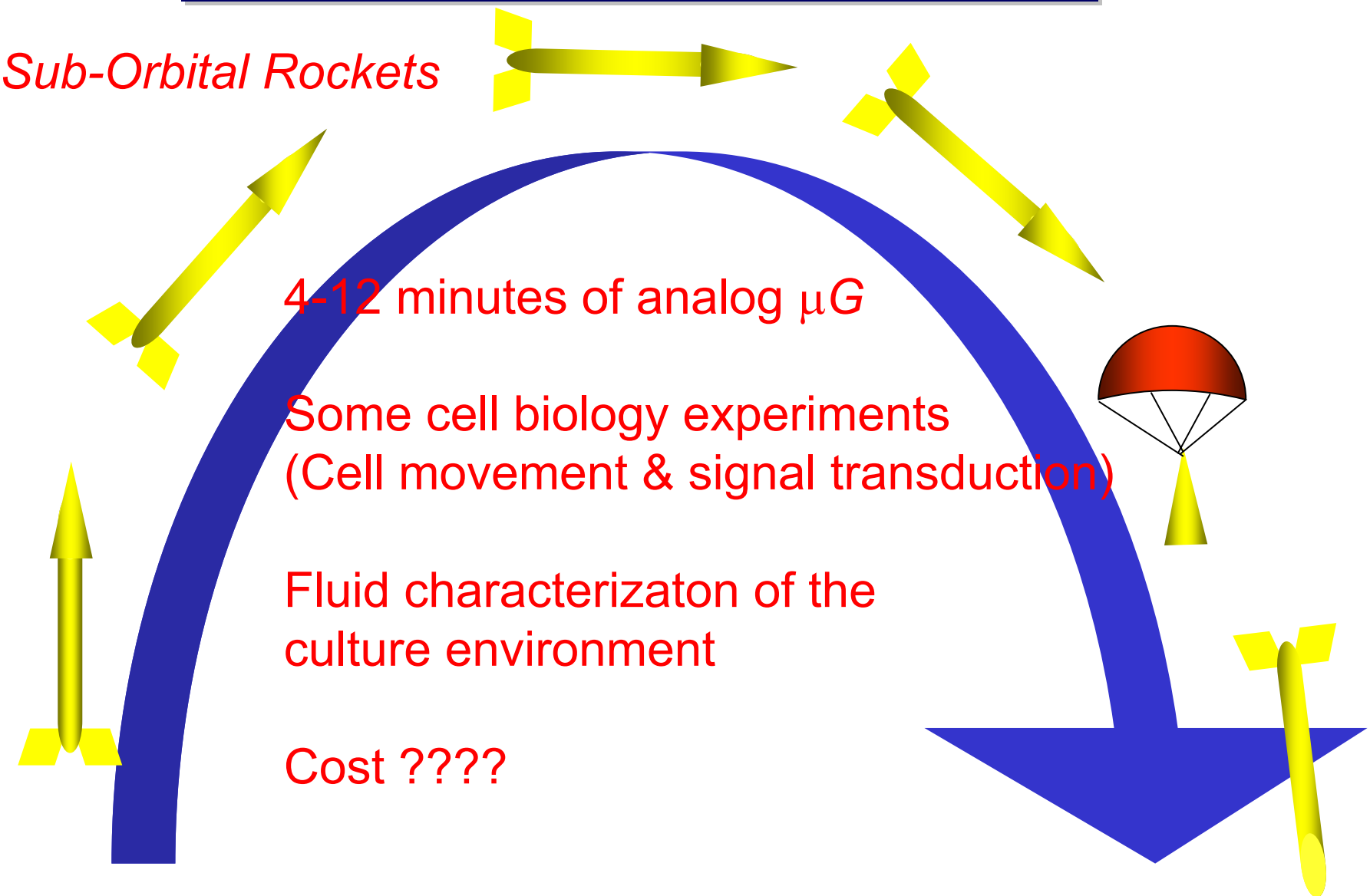
Too short of μG time for many cell biology experiments

Hyper *G* may confound interpretation of the data



Microgravity Analogs

Sub-Orbital Rockets



4-12 minutes of analog μG

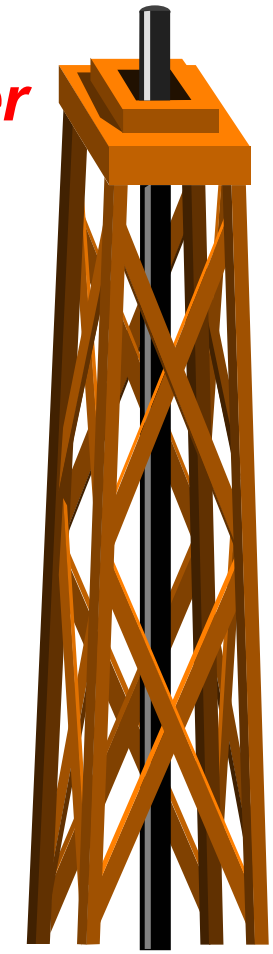
Some cell biology experiments
(Cell movement & signal transduction)

Fluid characterizaton of the
culture environment

Cost ????

Microgravity Analogs

Drop tower



5-60 seconds of analog μG

Difficult for many cell biology experiments
Better for fluid characterization of the culture environment

Cheap!!!

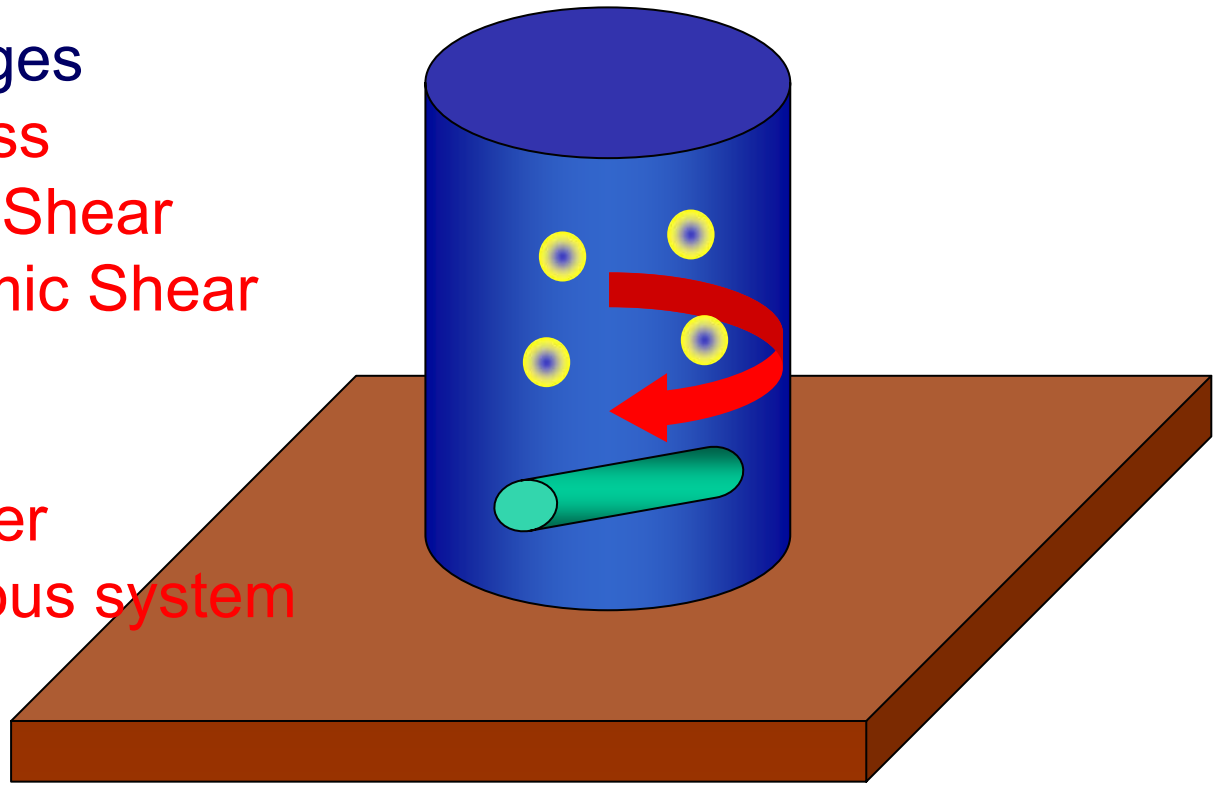
Amenable to repeat experiments

Suspension Strategies

Stirred Bioreactor

Disadvantages
Impact Stress
Mechanical Shear
Hydrodynamic Shear

Advantages
Mass transfer
Homogeneous system

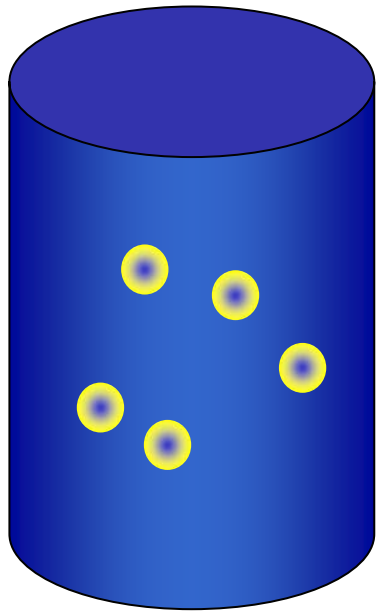


Suspension Strategies

Isopycnic Solution
(Neutral Buoyancy)

Disadvantages
Cells are at rest
Poor mass transfer
Poor tissue morphogenesis

Advantages
No sedimentation

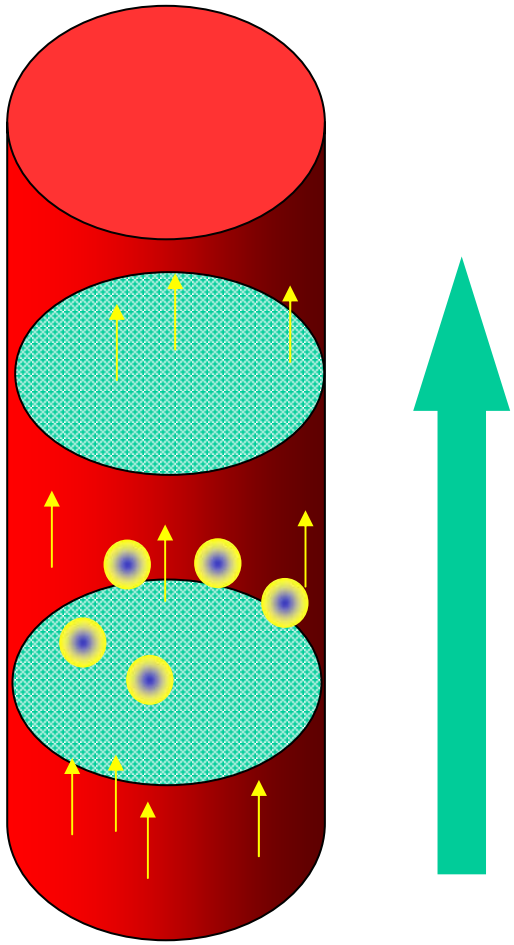


Suspension Strategies

Fluidized Bed

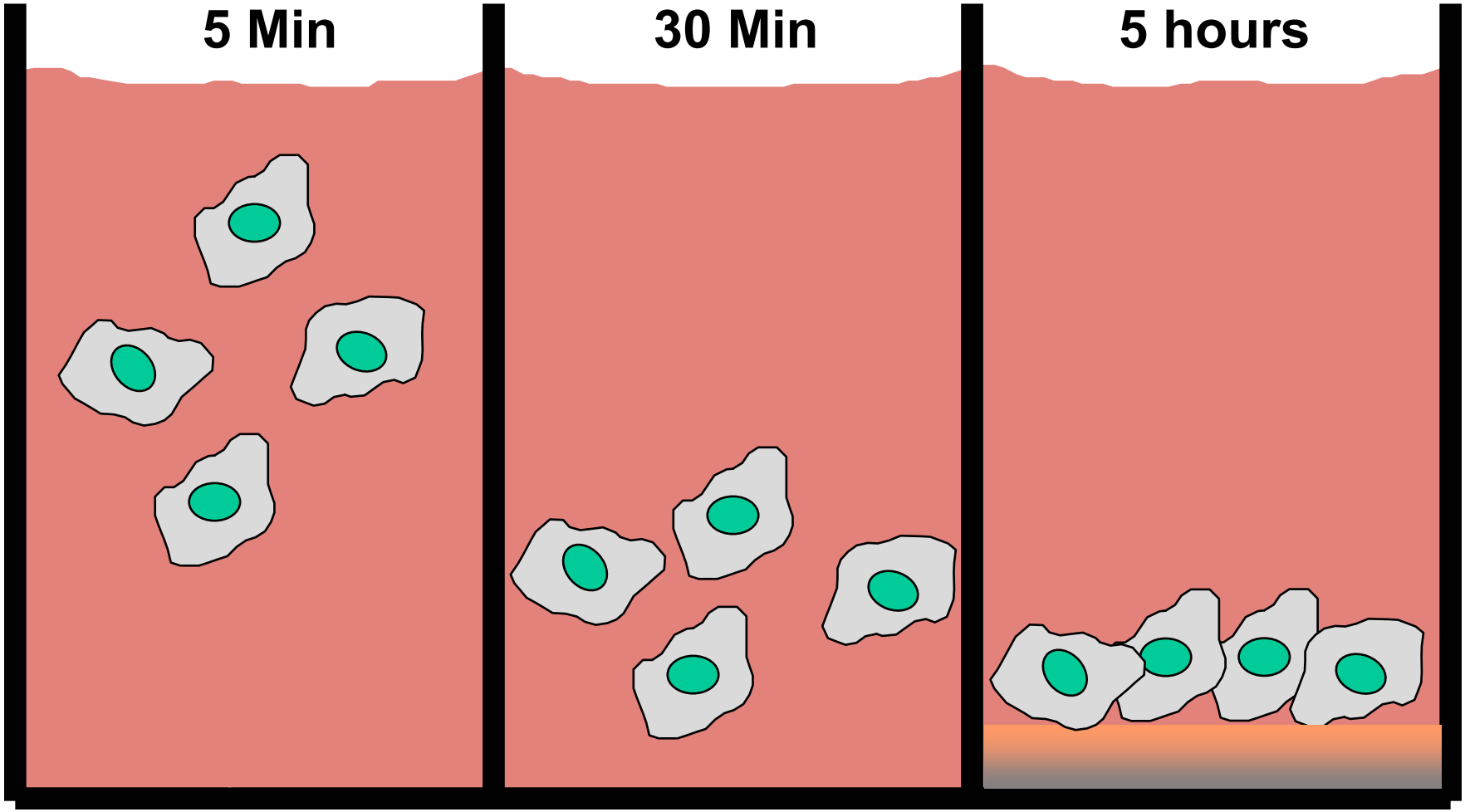
Disadvantages
Variable flow rates
Increasing hydrodynamic Shear
Very unstable

Advantages
Invokes free fall



Flow rate=Sedimentation rate

Prevention of cell attachment



Disadvantages

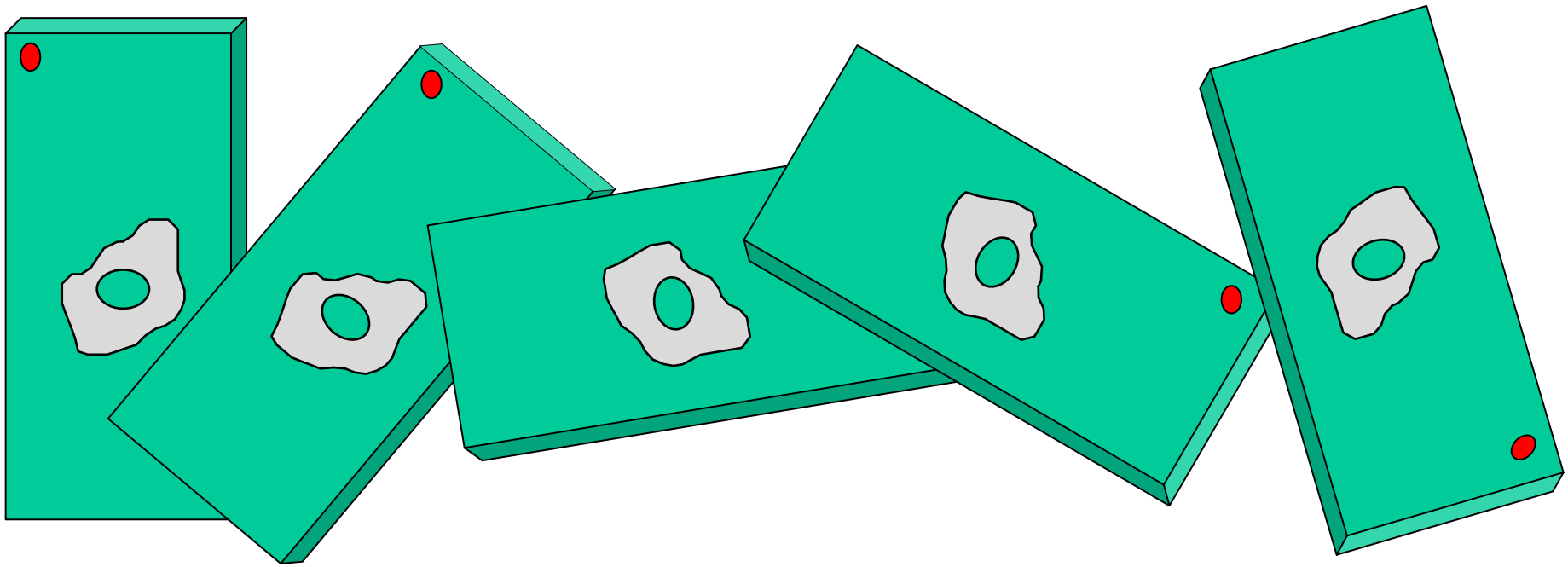
- Cells are still sedimented to a surface
- Many cells are unable to thrive
- Difficult to relate to microgravity

Advantages

- Easily achieved
- Useful in the analysis of the role of adherence

Analog Cultures

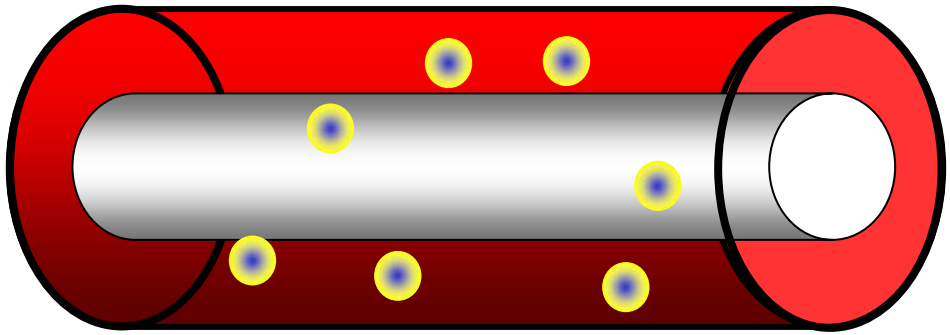
Clinostatic Rotation



- Cells adherent to a surface are rotated on one axis
- Randomized *G*

Analog Culture

Solid Body Fluid Rotation



Basis of operation

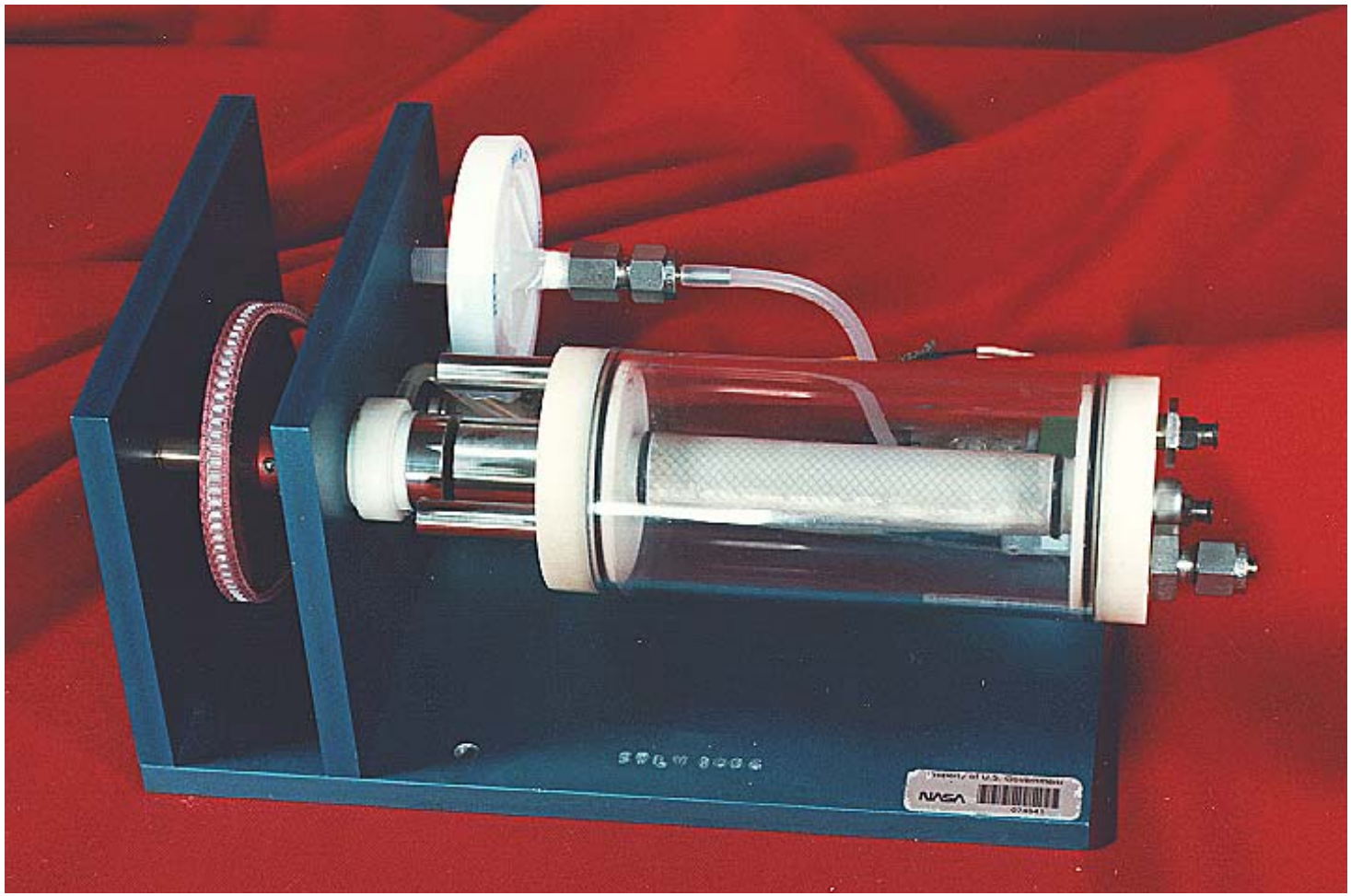
Zero head space

Fluid rotates with the cylinder

Results in particle suspension without stirring

Derivative of clinostatic rotation

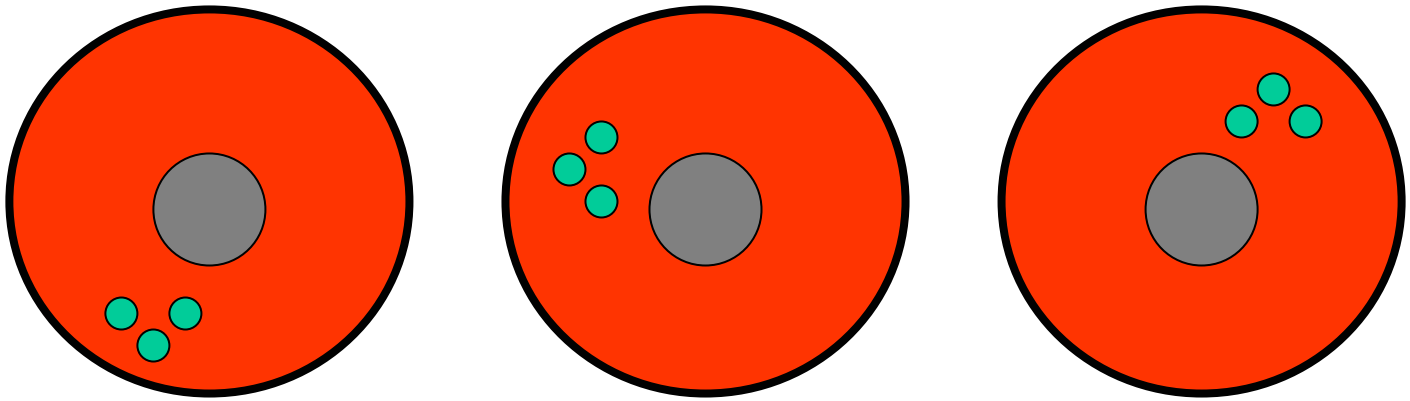
NASA Rotating Bioreactor



Licensed to Synthecon, Inc.

Microgravity Cell Culture Analog

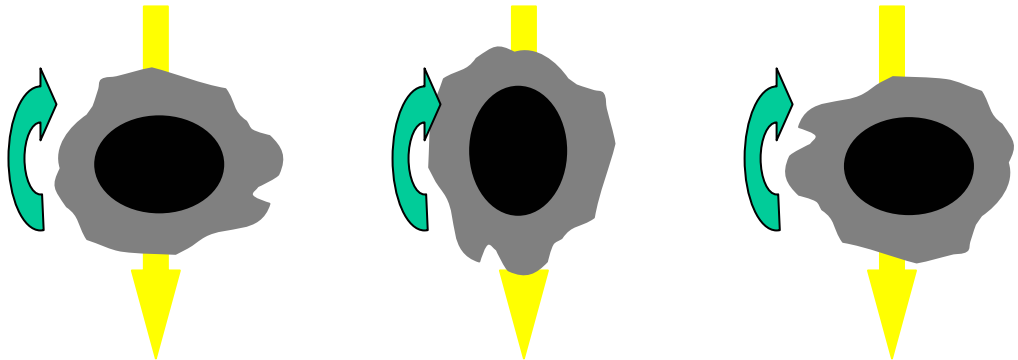
Solid Body Fluid Rotation



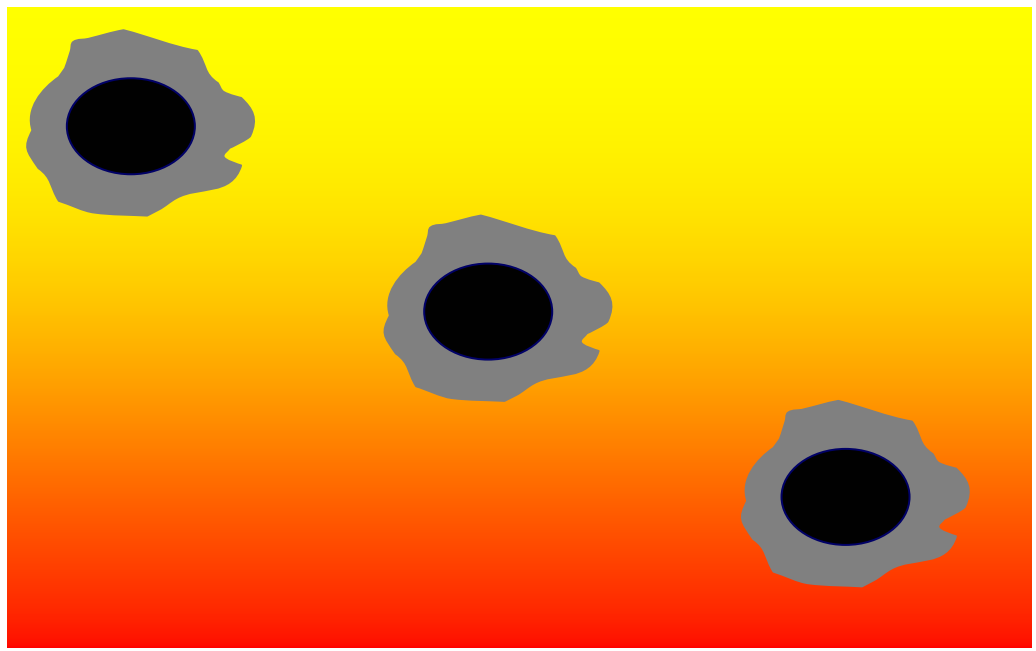
Fluid rotates with the cylinder
Cells are in near continuous suspension
Fluid shear is minimal (0.3 dynes/cm^2)
Suspension is possible for tissue assemblies ~0.5-1.0 cm

Microgravity Cell Culture Analog

Randomized *G*



Terminal Velocity Sedimentation



Advantages

- Suspension with minimal mechanical shear
- Low hydrodynamic shear
- Promotes tissue morphogenesis
- Sufficient mass transfer to support cell metabolism and growth
- May share some characteristics with space flight culture

Disadvantages

- Difficult to establish an appropriate control
- Multiple effectors responsible for results

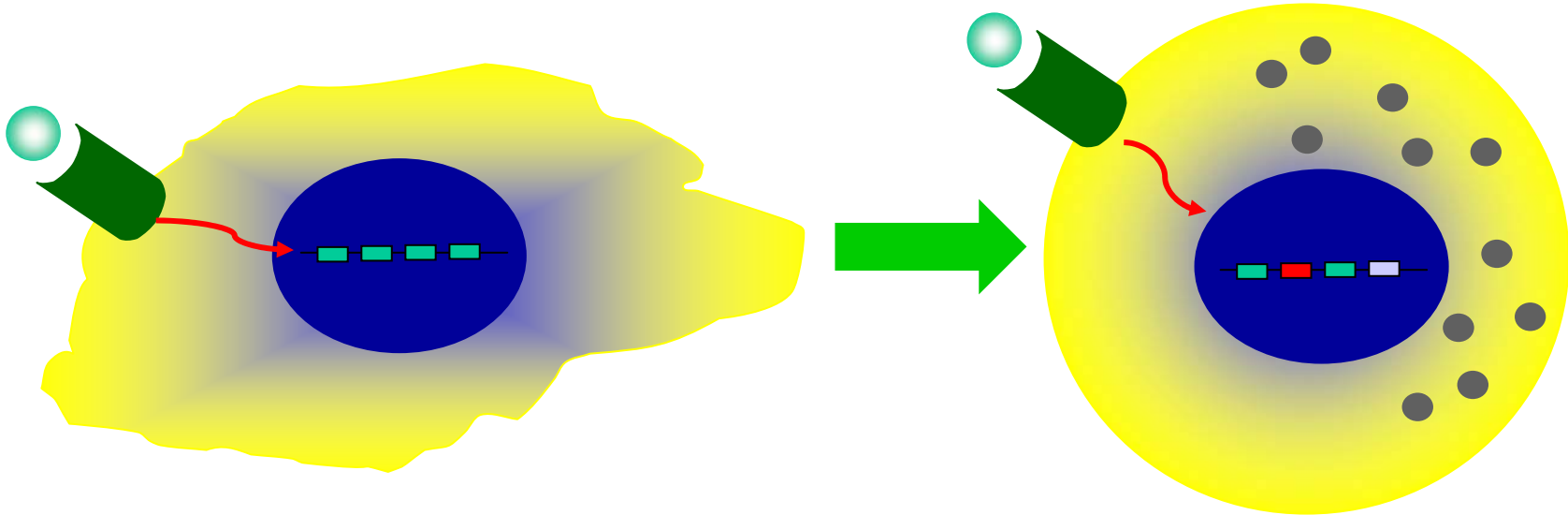
Results from Solid Body Rotation Culture

- 3-D propagation of tissue
- Greater frequency of successful co-culture
- May favor differentiation
- Models some aspects of cell function in microgravity
- May increase the ability propagate otherwise ‘difficult’ cells
- Production of novel biomolecules

Cellular Responses to Microgravity

1 *G*

μ *G*



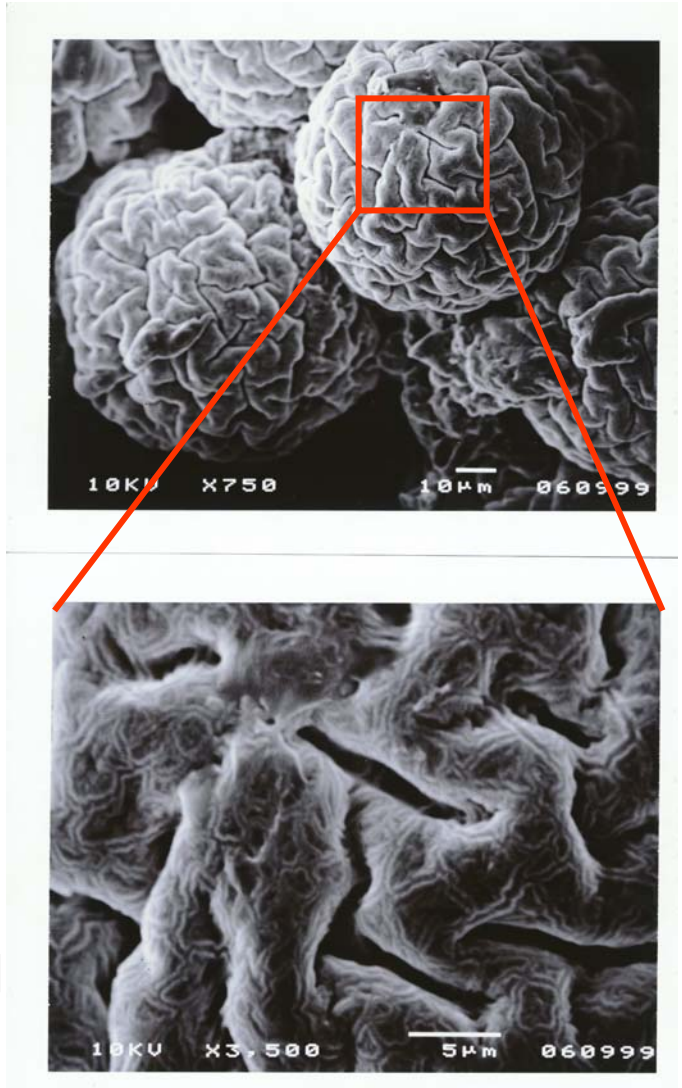
Change in fluid distribution
gene expression
signal transduction
locomotion
differentiation

Propagation of ‘Difficult’ Cells

The top photo show the first continuous cultures of Bowhead whale kidney cells growing on spherical microcarriers from the NASA bioreactor.

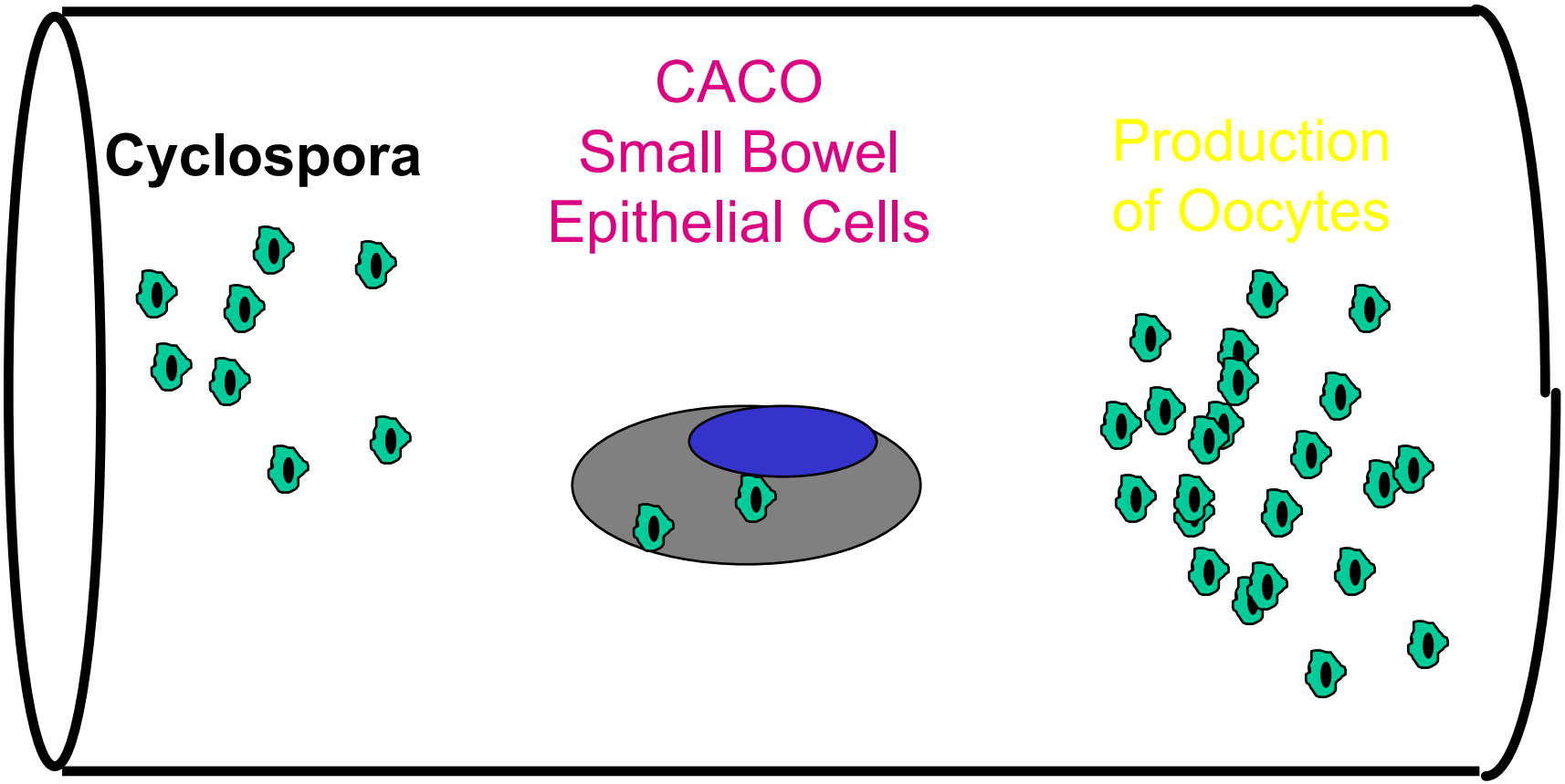
The lower photo is a magnification of the indicated area showing the unique features of the tissues from this species.

Scientists will use these cells to investigate the response of mammals to environmental toxins in marine ecosystems. These toxins can find their into the food chain not only affecting food sources but also the end user, humans.



Propagation of Protozoans

FDA /NIH/NASA-- Cyclospora



Physical Principles in Space Biology

Bacteria

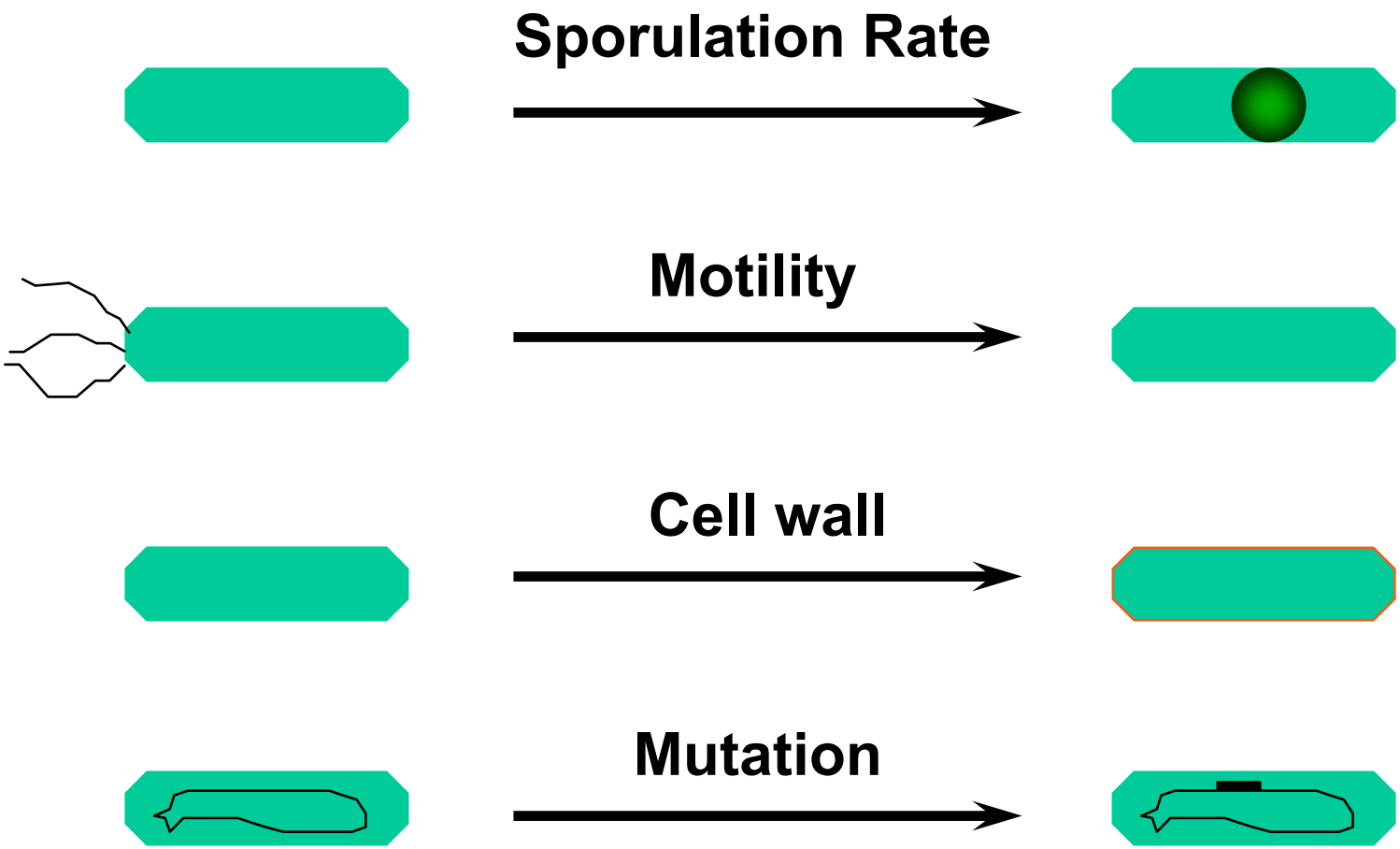
1 *G*

μ *G*

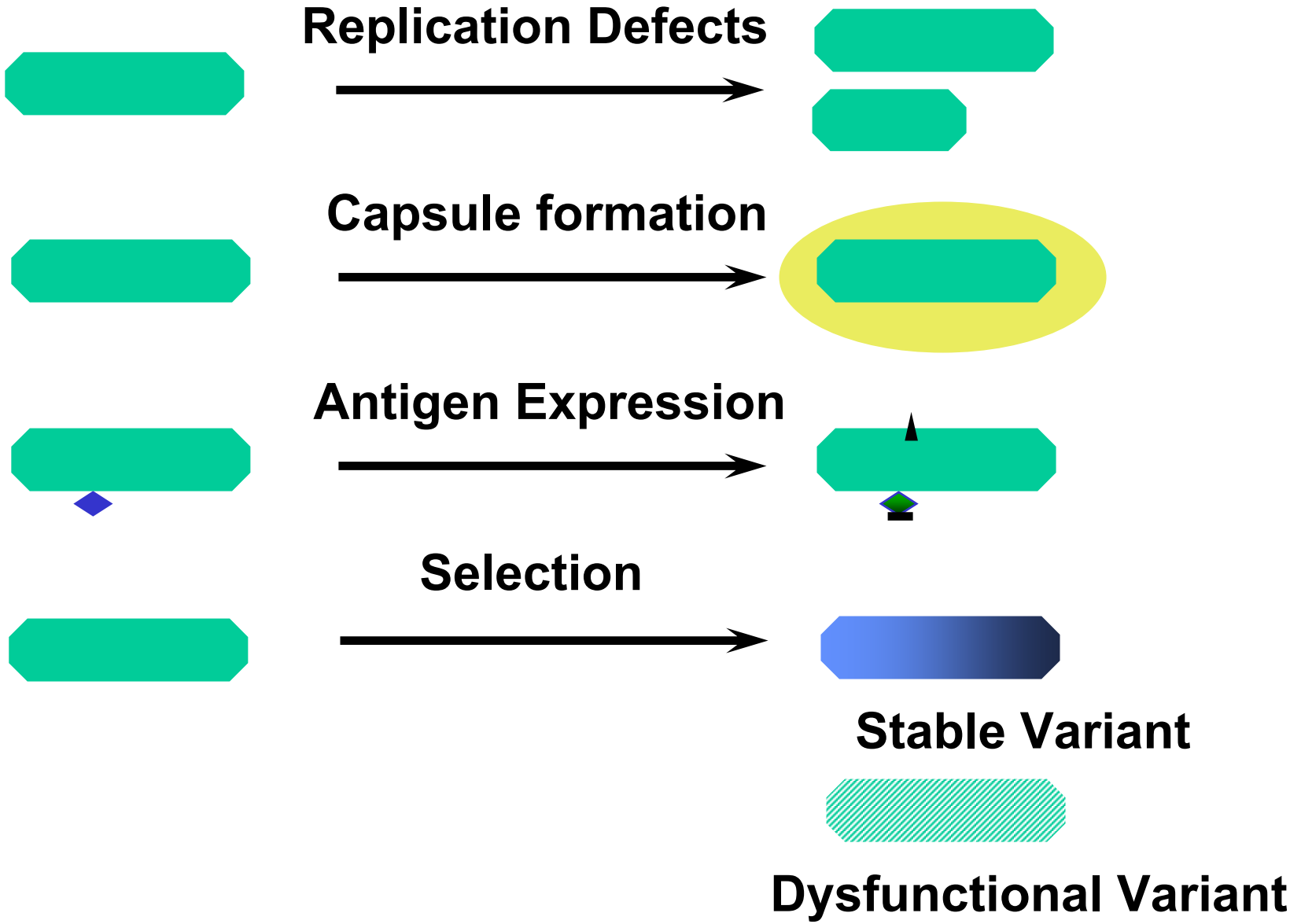
$P_{in} << P_{out}$

- Change in gene expression
- Chance in secondary metabolism
- Change in virulence

Using Microbes



Using Microbes



Microbes in Space

- Little is known
- Increased secondary metabolism
- Increased secretion
- Changes in virulence
- Biofilms
- Degradation of hydrocarbons

Significance

There is little doubt that cells respond decreased gravity environments. The mechanism of gravity induced responses in cells is unknown. Nevertheless, microgravity affords a unique tool to probe the underlying mechanisms in cell biology and to use the tool in novel ways to achieve goals in applied biological science and development.

Physical Factors to Consider in Experimental Design

- Gravity
- Mechanical impacts
- Hydrodynamic shear
- Convection
- Vibration
- Radiation
- Barometric Pressure

Module 1

- Novelty of the μG environment for cells
- Modeling μG for cells
- Trans membrane signalling
- Differentiation
- Gene expression
- Tissue engineering
- Disease modeling

Readings

- Cogoli, A and Gmunder, F.K. (1991) Gravity Effects on Single Cells: Techniques, Findings, and Theory. Adv. Space Biol. Med. 1:183.

- Unsworth, B.R. and Lelkes, P.I. (1998) Growing tissues in microgravity. Nat.Med. 4:901.